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REVIEW

Central tolerance: what have we learned from mice?

Tom M. McCaughtry · Kristin A. Hogquist

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Abstract Producing a healthy immune system capable of defending against pathogens, while avoiding autoimmunity, is dependent on thymic selection. Positive selection yields functional T cells that have the potential to recognize both self and foreign antigens. Therefore, negative selection exists to manage potentially self-reactive cells. Negative selection results from the induction of anergy, receptor editing, clonal diversion (agonist selection), and/or clonal deletion (apoptosis) in self-reactive clones. Clonal deletion has been inherently difficult to study because the cells of interest are undergoing apoptosis and being eliminated quickly. Furthermore, analysis of clonal deletion in humans has proved even more difficult due to availability of samples and lack of reagents. Mouse models have thus been instrumental in achieving our current understanding of central tolerance, and the evolution of elegant model systems has led to an explosion of new data to be assimilated. This review will focus on recent advances in the field of clonal deletion with respect to three aspects: the development of physiological model systems, signaling pathways that lead to apoptosis, and antigen presenting cell types involved in the induction of clonal deletion.

Keywords Thymus · Clonal deletion · Tolerance

The prevention of autoimmunity is primarily the outcome of central tolerance, which is achieved when thymocytes with high affinity for self-peptide/major histocompatibility complex (self-p/MHC) undergo negative selection. Four outcomes of negative selection have been described. First, receptor editing is a process by which thymocytes with high affinity for self-p/MHC are instructed to generate a second rearrangement of the T cell receptors (TCR)α loci, thereby altering the specificity of the TCR [1–4]. Secondly, anergy, or a state of induced unresponsiveness, has also been described [5]. The relative contribution of receptor editing and anergy to central tolerance is thought to be minimal. Instead, clonal diversion (agonist selection) of high-affinity thymocytes into lineages that attain immunoregulatory function is very important (reviewed in [6–9]). Finally, clonal deletion (induction of apoptosis in self-reactive clones) is the predominate mechanism by which central tolerance is achieved and will be the focus of this review.

T. M. McCaughtry · K. A. Hogquist (✉)
Center for Immunology, Laboratory Medicine & Pathology,
University of Minnesota,
Mayo Mail Code 334, 420 Delaware Street SE,
Minneapolis, MN 55454, USA
e-mail: hogqu001@umn.edu

Model systems

In 1957, Sir Macfarlane Burnet first proposed the concept of "repertoire purging" as a mechanism of lymphocyte tolerance [10]. This process was first described experimentally for thymocytes by studying clonal deletion in response to superantigens [11] (see Table 1). Superantigens are molecular remnants of proviruses in the murine genome that cross-link particular Vβ segments of TCRs with class II MHC. While this mimics a high-affinity TCR ligation, it is unclear if the downstream signaling events are entirely the same as stimulation by p/MHC. Soon after, in vitro stimulation of thymocytes with high-affinity ligands was utilized to model clonal deletion [12–14]. However, it is unlikely that in vitro stimulation recapitulates the in vivo process of clonal deletion because the intact thymic microenvironment is missing. The switch to in vivo models was a substantial improvement and first utilized the injection of TCR-cross-linking antibodies to simulate

Table 1 A summary of model systems used to study negative selection

Model system	Caveats	Advantages	Disadvantages
Superantigens	Only particular Vβ TCR are deleted	Endogenous antigen	Independent of TCR affinity for p/MHC
	Expression mostly in medulla		TCR signal may be qualitatively or quantitatively distinct
In vitro αCD3/αCD28 TCR Tg peptide	Costimulation required	Technically simple	Intact thymic microenvironment lacking
In vivo: αCD3/αCD28		Intact in vivo environment	Activation of peripheral T cells causes nonspecific deletion. Glucocorticoid mediated
In vivo: TCR Tg with injected peptide		Intact in vivo environment	Activation of peripheral T cells causes nonspecific deletion. Cytokine mediated
In vivo: TCR Tg with transgenic neo-self antigen		Intact in vivo environment	Early TCR expression TCR expression level higher than normal on thymic precursors High precursor frequency/monoclonal Transgenic-antigen artifacts (expression pattern/level)
In vivo: TCR Tg with endogenous self-antigen		Intact in vivo environment	Early TCR expression TCR expression level higher than normal on thymic precursors High precursor frequency/monoclonal
In vivo: TCR Tg mixed bone marrow chimeras with endogenous self-antigen		Intact in vivo environment Precursor frequency is lower	Early TCR expression TCR expression level higher than normal on thymic precursors
In vivo: "On-time" TCR Tg with endogenous self-antigen		Intact in vivo environment Appropriate TCR timing (can use in mixed chimeras to obtain low precursor frequency)	TCR expression somewhat higher than normal on thymic precursors
In vivo: Vβ transgenics with endogenous self or neo-self antigens		Intact in vivo environment Appropriate TCR timing and level Precursor frequency is lower/oligoclonal	Inability to track cells prior to selection in DP thymocytes

high-affinity TCR ligation [15]. However, it was more recently observed that activation of peripheral T cells and their subsequent release of pro-inflammatory cytokines and stress hormones [16] could be obscuring TCR-induced death of DP thymocytes. The development of TCR transgenic mice was a major advancement in the field of thymic development and allowed researchers to examine negative selection in response to true p/MHC stimulation. Some studies have utilized exogenous administration of peptide to induce negative selection [17], but this also has the caveat of activation of peripheral T cells [18, 19]. A more physiological model has been to examine the response to endogenous high-affinity peptides, either produced transgenically (neo-self-antigens) [4, 20, 21] or naturally occurring [22, 23].

The use of different model systems has led to conflicting conclusions about the developmental stage at which thymocytes undergo clonal deletion. For example, superantigen studies have suggested that deletion occurs at the single positive (SP) stage, whereas the examination of TCR transgenics and endogenous self-antigens have suggested that deletion occurs at the transition from double negative (DN) to double positive (DP). This apparent discrepancy can be partially rectified by observation that superantigens are primarily expressed in the medulla, which is the site where SP thymocytes reside. Furthermore, the nature of transgenic TCR expression has called into question the deletion observed at the DN to DP transition. Wild type thymocytes rearrange their TCRβ loci at the DN stage and if successful, they transition to the DP stage and commence rearrangement of the TCRα loci. Thus, thymocytes are not competent to undergo deletion until they have expressed a heterodimeric TCRαβ at the DP stage. However, TCR transgenic thymocytes express both TCRα and TCRβ chains early at the DN stage and probably undergo negative selection prematurely, which could explain the observation that deletion happens at the DN to DP transition. It is clear that the nature of TCR and self-antigen expression can dramatically impact the timing of clonal deletion and perhaps the molecular mechanism by which apoptosis is induced. It is therefore important to move forward by making use of the most physiological tools available.

As an example, our laboratory recently generated a TCR transgenic mouse that recapitulates the appropriate timing of TCRα expression at the DP stage (the HY^{cd4} model) [24]. HY^{cd4} thymocytes bear the same TCR as the original HY transgenic mouse made by Harold Von Boehmer's laboratory [23] and has a high affinity for a self-peptide derived from the Y chromosome in male mice, but because of the appropriately timed expression of the HY TCRα chain at the DP stage, they undergo deletion at the DP to SP transition. This is in stark contrast to when the TCRα chain is expressed prematurely in DN thymocytes and deletion occurs at the DN to DP transition (either in the conventional HY mouse [23] or when we utilized lck-driven Cre [24]). Other studies have utilized TCRβ-only transgenic mice to eliminate nonphysiologic artifacts of transgenic TCRα expression [21, 25]. This approach has the added benefit of producing an oligoclonal repertoire with reduced precursor frequency of antigen-specific thymocytes, which also corrects defects in thymic architecture that are typical of conventional TCR transgenics [26, 27]. The latter study by Gallegos and Bevan also made use of the unique expression of tissue-restricted antigens (TRAs) in the medulla (explained in more detail below). This system obviates the artifacts introduced by premature transgenic TCR expression because only postpositive selection thymocytes, transitioning from the DP to SP stage and migrating to the medulla, are encountering high-affinity antigen and undergoing deletion (See Fig. 1). One lingering defect of all TCR transgenic models is the expression level of the TCR transgenes. Wild-type DP thymocytes express very little TCR on their cell surface and upregulate its expression following positive selection, whereas TCR transgenes driven off of strong promoters express at higher levels. Unfortunately, creating a TCRα knock-in into the endogenous locus still bears this artifact [28], although a TCRβ knock-in has not yet been created. Thus, a model for completely physiological transgenic TCR expression remains elusive. Future studies examining negative selection should strive to make use of models that have appropriately timed TCR expression, at physiological levels, with high affinity for a physiologically expressed, endogenous self-antigen. Precursor frequency of antigen-specific cells should also be minimized. Ideally, the analysis of a polyclonal repertoire should be employed; however, it is currently difficult to identify small numbers of precursors, due to the fact that DP thymocytes express low levels of TCR and are not easily distinguished by p/MHC tetramer staining.

Molecular mediators of clonal deletion

A fundamental question of T cell development has been: How does a thymocyte distinguish between positive and negative selection ligands? The pervasive "affinity model" predicts that the kinetics of TCR binding to self-p/MHC determines this outcome. There is much support for this model, and recently, the precise threshold for this distinction based on affinity was determined for class I MHC-restricted TCRs [29]. Nonetheless, how a cell translates TCR affinity into the decision between life and death is less well understood. It has been suggested that high-affinity TCR ligation induces a conformational change in the cytoplasmic tail of CD3ε, which exposes a polyproline sequence that serves as a binding site for the adapter protein Nck [30]. However, another study suggested that low-affinity interactions also expose the polyproline sequence, albeit less efficiently [31]. Recently, Mingueneau and colleagues created knock-in mice with a mutation in the CD3 polyproline region. Surprisingly, they found that binding of Nck does not depend on previous TCR ligation. Additionally, positive selection was affected by introduction of the mutation in two TCR transgenic models, but negative selection was not [32]. The misshapen-Nck-interacting kinase-related kinase (MINK) was also suggested to be important for the induction of negative selection [33], although this finding has not further corroborated. With respect to TCR-proximal signaling events, it has been suggested that differential activation of the extracellular signal-regulated kinase (ERK) and *c*-Jun N-terminal kinase (JNK) pathways is important in this discrimination (discussed in depth in [34]). A recent report extended these findings by demonstrating the differential subcellular localization of Ras and mitogen-activated protein kinase signaling intermediates in response to positively and negatively selecting ligands [35]. Thus, it appears that Ras-mediated activation of the ERK pathway is dispensable for clonal deletion. Interestingly, Ras signaling is not only involved in positive selection of conventional T cells [36] but also in the generation of $Foxp3^+$ Tregs [37], which seem to be selected by high affinity ligands [6]. This may be important for the decision between clonal deletion and clonal diversion. It was also recently shown that deficiency of the protein phosphatase G5PR resulted in hyperactivation of JNK and caspase 3 [38], indicating that G5PR inhibits apoptosis in the steady state and that overcoming this inhibition by G5PR is perhaps necessary for clonal deletion. A role for costimulation to induce clonal deletion has also been suggested. However, this has been controversial, likely due to use of different model systems (see [34] for a thorough review). While αCD28 strongly costimulates apoptosis in vitro, no single costimulatory molecule has been shown to be required in all models of clonal deletion. It is possible that multiple distinct costimulatory molecules function redundantly in clonal deletion.

The signals leading to clonal deletion ultimately activate the apoptosis pathway (see [39] for a detailed review), and a number of groups have implicated the orphan nuclear

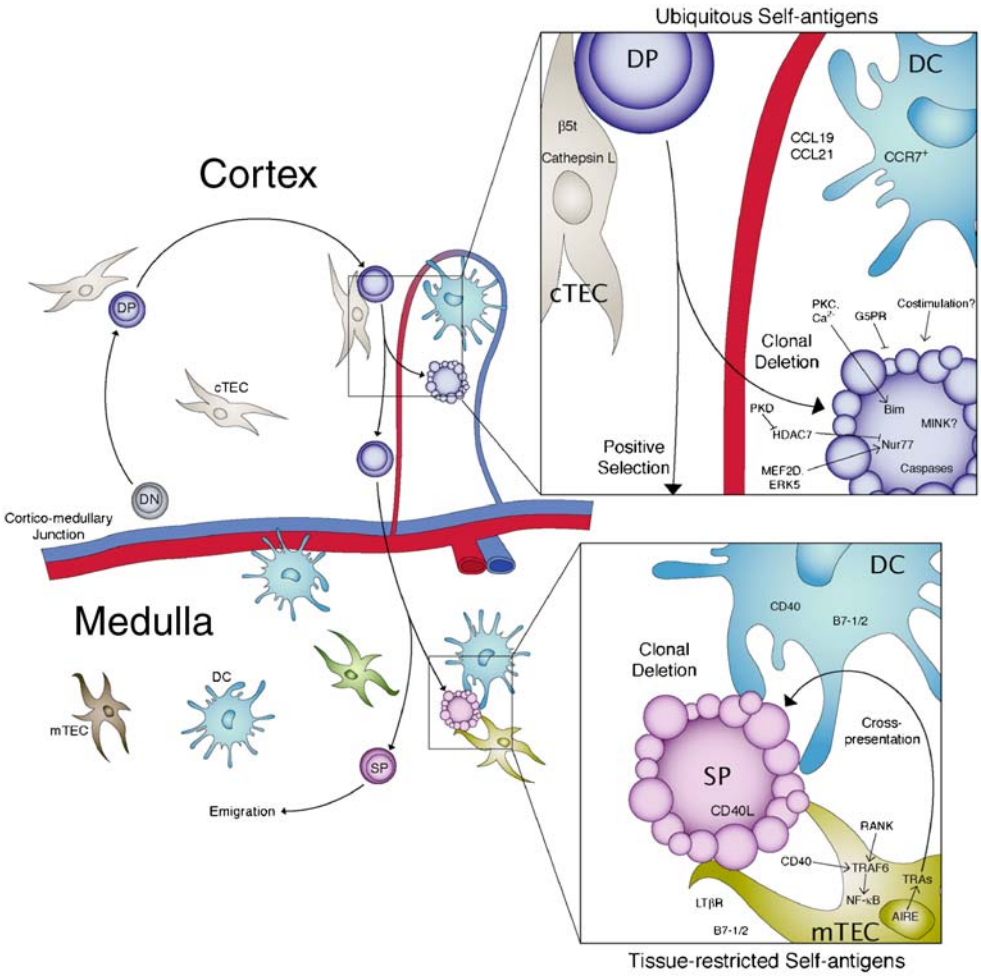

Fig. 1 Distinct stages of clonal deletion in the thymus. DP thymocytes are positively selected in the thymic cortex when they interact with cortical thymic epithelial cells (cTEC). cTEC themselves are likely not efficient inducers of clonal deletion. However, DP thymocytes that are triggered through the TCR upregulate CCR7. This facilitates their movement toward the thymic medulla. It may also facilitate interaction with dendritic cells (DC) in the cortex (upper right box) which produce CCR7 ligands. Cortical dendritic cells are required for efficient clonal deletion of those DP thymocytes that recognize ubiquitious self-antigens. Thymocytes that recognize tissue-specific self antigens are deleted later, at the SP stage in the thymic medulla (lower right box). Medullary thymic epithelial cells (mTEC) produce tissue specific self-antigens in an AIRE dependent fashion, and can directly present to SP thymocytes. Dendritic cells, which are abundant in the medulla, can also cross present antigens for clonal deletion

steroid receptor Nur77 as being necessary [40, 41]. It was shown using cultured thymocytes that myocyte enhancer-binding factor 2D (MEF2D) and ERK5 mediate transcription of Nur77 [42]. On the other hand, Nur77 expression is repressed by histone deacetylase 7 (HDAC7), and this inhibition can be overcome by TCR stimulation that activates protein kinase D1 which subsequently phosphorylates HDAC7 and promotes its nuclear export [43–45]. While it has been well documented that Nur77 is induced following a strong TCR stimulation, it is less clear how Nur77 exerts its pro-apoptotic function. It was first proposed that Nur77 acts a transcription factor to promote apoptosis [46], but a more recent report suggested that Nur77 mediates apoptosis by translocating to the mitochondria where it sequesters the anti-apoptotic molecule Bcl-2 [47]. It remains unclear if the role of Nur77 as a transcription factor is also necessary. Defining the precise role for Nur77 in negative selection has been further complicated by the observation that Nor-1, a closely related family member, can have functional redundancy with Nur77 [48].

The pro-apoptotic molecule Bim also plays a central role in clonal deletion [49]. The precise mechanisms leading to Bim induction have not been fully elucidated, although Ca^{2+} and protein kinase C were suggested to be important [50]. A number of recent reports have investigated the transcriptional profile of thymocytes undergoing negative selection [51–55]. The most recent of these, by Baldwin and Hogquist, compared the gene array data sets generated by the different groups and found surprisingly little overlap between them. Specifically, only Bim, Nur77, PD-1, and Gadd45β were consistently found to be upregulated. This might suggest that clonal deletion only requires a small number of genes to be newly synthesized. However, it has yet to be demonstrated that new transcription of any of these genes after the initial TCR ligation is absolutely necessary for deletion. It has been suggested that transcription of Bim is required [50], but it has also been argued that phosphorylation of Bim is the critical event in promoting clonal deletion [56].

Finally, an intriguing report recently suggested that posttranscriptional regulation of mRNA by microRNAs might be important in the distinction between positive and negative selection [57]. Li et al. showed that miR1–181a acts as an intrinsic "rheostat" to control T cell sensitivity in thymocytes and mature T cells. It is likely that miR-181a plays a role in

setting the bandwidth for discrimination of positive and negative selection signals, and perhaps the regulation of miR-181a itself or its targets plays a role in inducing apoptosis following a high-affinity TCR signal. Our understanding of the role of microRNA in T cell development is in its infancy and is likely to be even more greatly appreciated in the future as evidenced by other recent work examining DICER and microRNA in T cell development and function [58–60].

Cell types that mediate clonal deletion

Cortical thymic epithelial cells

Thymocytes interact with a variety of antigen-presenting cells during the course of their development. DN thymocytes enter the thymus at the corticomedullary junction (CMJ) and traverse outwards towards the subcapsular region where they undergo rearrangement of the TCRβ chain (Fig. 1). As DPs, they migrate randomly though a dense matrix of cortical thymic epithelial cells (cTECs), and their engagement with p/MHC on cTECs is crucial for positive selection [61]. Whether or not cTECs are also capable of inducing negative selection is less clearly understood. A number of studies have concluded that cTECs are not capable of inducing tolerance to self-antigens [62–66]. However, there is also much data to suggest that cTECs can in fact be tolerogenic [4, 67–75]. This apparent contradiction is likely explained by the observation made by Goldman et al. that studies indicating the thymic epithelium was not capable of inducing tolerance "were done using antigens not normally expressed by thymic epithelium and/or targets derived from other tissues". They then go on to suggest that cTECs are capable of inducing tolerance to antigens expressed by cTECs, but not to all antigens expressed in the body. In addition, we have shown that cTECs are inefficient at inducing apoptosis of self-reactive thymocytes but that they are ultimately tolerogenic because they prevented the development of mature SP thymocytes [76]. We were unable to determine if cTECs induced anergy or clonal diversion of these cells, but these observations may also help reconcile the apparent contradictions in previous studies because the induction of tolerance can occur by mechanisms other than clonal deletion.

An intriguing study was recently reported that potentially changes the paradigm of cTECs in positive and negative selection [77]. In addition to the "standard" proteasome and the "immunoproteasome", Murata et al. described a novel proteasomal subunit expressed exclusively in cTECs, which they termed β5t. This "thymoproteasome" has unique peptidase activity with reduced chymotrypsin-like activity, which is important for generating peptides with hydrophobic C-termini. Hydrophobic C-termini anchor peptides in the groove of class I MHC. Therefore, it is likely that cTECs not only generate a unique peptide repertoire but also present unstable class I MHC on their cell surface. These observations may explain why cTECs are critical for positive selection, and in fact, β5t-deficient mice had a dramatic defect in generation of CD8 SPs. This unique feature of cTECs also potentially explains why cTECs are incapable of inducing tolerance to antigens expressed throughout the body and their poor ability to induce clonal deletion. It is interesting to speculate that a similar mechanism exists in cTECs for the generation of class II p/mHC complexes, perhaps via cathepsins [78, 79].

Trafficking to the medulla

The notion that cTECs are not sufficient for induction of clonal deletion is bolstered by the observation that trafficking to the medulla is necessary for achieving complete tolerance [80, 81] and that this migration is primarily mediated by CCR7-dependant signaling [82, 83] (See Fig. 1). Indeed, mice with medullar defects also display autoimmune phenotypes (reviewed in [84]). It is commonly believed that the medulla is a specialized anatomical location for clonal deletion due to the high density of dendritic cells (DC) and the peculiar ability of medullary thymic epithelial cells (mTECs) to ectopically express TRAs (both of which are described in further detail below). However, a defect in clonal deletion may not be sufficient to explain the lack of tolerance in previously mentioned studies. This is because the medulla has also been implicated to be important for the generation of immunoregulatory T cells [85–88] and for conventional T cells to undergo final maturation and adjustment of their TCR sensitivity to self-antigens (a process referred to as "TCR tuning") [89]. In addition, Reinhold Förster's laboratory also questioned whether or not CCR7 was the only chemokine receptor involved in corticomedullar migration [90]. While there is clearly medullar dysplasia in CCR7-deficient mice and reduced medullar volume, they showed that proportionally normal numbers of CD4 SP thymocytes were found in the small pockets of medulla that remained. They concluded that a redundant mechanism exists for migration to the medulla. Importantly though, it should be noted that the majority of SPs in the medulla were CD4 SPs, and it was impossible to distinguish if these were truly developing thymocytes or another lineage of $CD4^+$ T cells, such as $Foxp3^+$ Tregs that also reside in the medulla [91] and may have an alternate homing mechanism.

Medullary thymic epithelial cells

Despite the complicating factors of Treg development, TCR tuning, and the role of CCR7-mediated chemotaxis, it is clear

that developing thymocytes must gain access to the medulla to be screened against a panel of self-antigens uniquely expressed there (See Fig. 1). It has been shown that medullary thymic epithelial cells are capable of expressing the autoimmune regulator (AIRE), which leads to the ectopic expression of tissue-restricted antigens [92, 93]. This phenomenon of mTECs is crucial for the establishment of central tolerance as AIRE-deficient humans and mice both develop autoimmunity [94]. It has been shown that mTECs express the costimulatory molecules B7-1 and B7-2 and that direct presentation of TRAs by mTECs is sufficient to induce clonal deletion of antigen-specific thymocytes, although cross-presentation by dendritic cells also occurs [21] (discussed in further detail below).

The development of mTECs is dependent upon a population of $CD4^+$ $CD3^-$ lymphoid tissue inducer cells and receptor activator of nuclear factor κB (RANK)–RANK ligand interactions, and their maintenance is, in part, controlled by CD40–CD40L signaling and lymphotoxin-β receptor (LTβR; see Fig. 1) [95–97]. Furthermore, tumor necrosis factor receptor-associated factor 6 (TRAF6), which signals downstream of RANK and CD40, is also needed for mTEC development [86]. TRAF6 activates the transcription factor NF-κB, which is also required for mTEC development [98], as is the NF-κB-inducing kinase [85] and IκB kinase α [99].

Recent work has also demonstrated that mTECs are a highly dynamic population with high proliferative capacity and a turnover rate of about 2 weeks [100, 101]. It was observed that the majority of proliferating mTECs were mature, UEA-1^+ MHC class II^{hi}, and that these mature mTECs are the ones expressing AIRE [102]. In addition, it appears that AIRE-expressing mTECs are short-lived with rapid turnover [103]. While AIRE-dependent TRAs are found clustered throughout the genome, implying a role for epigenetics in AIRE function [104], it has also been shown that individual mTECs express a stochastic battery of TRAs, such that each mTEC is unique with respect to its antigen-presentation profile [105, 106]. Taken together, these data paint a picture where mTECs are critical for the elimination of self-reactive thymocytes and that individual AIRE-expressing mTECs are highly diverse and turning over rapidly. As loss of tolerance to just one tissue-restricted antigen is sufficient to cause autoimmunity [107], it becomes clear that thymocytes must have ample opportunity to peruse the entire milieu of the medulla to be adequately screened for self-reactivity. We recently showed that the length of time that an SP thymocyte spends in the medulla is roughly 4–5 days [108], during which a thymocyte "tunes" its TCR and becomes refractory to apoptosis. Therefore, a short medullary residency time of SP thymocytes, in combination with the diversity and turnover of mTECs expressing TRAs, may be a weak point in the mechanism of central tolerance. Evolution has obviously created a system that prevents overt autoimmunity but bear in mind that T cell-driven autoimmunity is disturbingly common in the human population. Finally, the precise mechanism by which AIRE operates is not entirely understood. AIRE most likely operates as a transcription factor, but as mentioned above with regards to epigenetics, it remains possible that AIRE promotes ectopic expression of TRAs by another mechanism(s) [109]. In addition, not all TRAs are AIRE-dependent [104]; thus, another mechanism or molecule, similar to AIRE, must also exist, perhaps involving LTβR [110].

Dendritic cells

Dogma states that TCR activation and clonal deletion are most efficiently induced by bone marrow-derived cells, and it is widely believed that dendritic cells are the principle mediator of clonal deletion. This is because DC are thought of as "professional" antigen presenting cells with high levels of class II MHC on the cell surface as well as high levels of costimulatory molecules, such as B7-1 and B7-2. Indeed, a direct role for DC in clonal deletion has been demonstrated [21, 111, 112]. However, the heterogeneity in thymic DC is largely underappreciated and not completely understood. It is unclear if all DC in the thymus are equal in their capacity to induce clonal deletion. Work by Donskoy and Goldschneider has shown that at least two distinct populations of thymic DCs exist: one that develops intrathymically and one that is derived from peripheral DCs migrating to the thymus [113]. It was suggested that these two populations might have functional differences in their ability to induce deletion of self-reactive thymocytes vs. clonal diversion of immunoregulatory cells. Goldschneider and Cone propose a model where intrathymically derived DC mediate clonal deletion, whereas extrathymically derived cells support the agonist selection of regulatory T cells (see [114] for a comprehensive review of this argument). It is important to note that the phenotype is unknown of the subset of peripheral DCs that are capable of migrating to the thymus and mediating this selection. In line with this hypothesis, it has been shown in the human thymus that a group of mTECs called Hassall's corpuscles produces TSLP (thymic stromal lymphopoietin) and this allows DC to induce the proliferation and differentiation of $Foxp3^+$ Tregs [115]. Conversely, it was more recently demonstrated that antigen-loaded, splenic DC were capable of homing to the thymus and inducing deletion of antigen-specific thymocytes [116]. More detailed analysis of the phenotype of the DC migrating to the thymus was also performed, and these DC included all subsets of splenic DC that were present at the time of injection, although LPS-matured DC showed a reduced capacity to home to the

thymus. It may be that the ability of peripheral DC to migrate to the thymus and induce deletion vs. agonist selection might not be mutually exclusive and may depend upon the specific environmental and experimental conditions. It is also possible that different subsets of peripheral DC are capable of mediating the different selection outcomes.

As potential support for the latter hypothesis, Ken Shortman's laboratory has shown that, in addition to intrathymically- and extrathymically derived DCs, at least three other DC subsets can be identified based upon cell surface markers (reviewed in [117]). Their data showed an initial division of DC into two populations, 35% of which are plasmacytoid DC (pDC) and the remaining 65% are conventional DC (cDC). The distinction between these two populations is based on expression of CD11c and CD45RA, with pDC being $CD11c^{int}$ and $CD45RA^{+}$, and cDC being $CD11c^{hi}$ and $CD45RA^{-}$. The majority of pDC are $Ly6c^{+}$, although a small fraction of $Ly6c^{-}$ can also be found. Similar to pDC in the spleen and lymph node, some thymic pDC can express CD4 and/or CD8α, but unlike peripheral pDC, they express high levels of TLR7 and 9, but only low levels of TLR 2, 3, and 4. Also similar to peripheral pDC, thymic pDC express low levels of MHC class II and costimulatory molecules and can take up antigen by endocytosis but not phagocytosis. Thus, pDC are weak stimulators of T cells. On the other hand, all conventional DC are MHC class II+ and the majority are $CD8\alpha\alpha^{+}$. Similar to the minority population of $CD8^{+}$ DC in the spleen and lymph node, this population in the thymus is DEC-205^{+} (CD205) and $CD11b^{-}$. However, unlike peripheral $CD8\alpha^{+}$ DC, some thymic $CD8\alpha^{+}$ cDC also express BP-1, although no functional difference has yet been shown based upon this distinction. Conventional DC can be further subdivided by their expression of CD8α and Sirpα (CD172a). $CD8\alpha^{lo}$ $Sirp\alpha^{+}$ DCs account for 20% of the thymic cDC population, and similar to splenic $CD8\alpha^{-}$ DC, this population is also $CD11b^{int}$. While thymic cDC express the costimulatory molecules B7-1 and B7-2 at slightly higher levels than their peripheral counterparts, for the most part, they appear to be immature because they are capable of phagocytic or endocytic uptake of antigens and process and present them on MHC class II. In addition, they upregulate expression of MHC class II and costimulatory molecules following activation. It is not entirely clear how this classification of thymic DC aligns with the division created by the Goldschneider laboratory as to the origin of these cell types. It seems that most of $CD8\alpha^{+}$ cDC develop intrathymically from a lymphoid prothymocyte precursor, whereas extrathymically derived DC include the $CD8\alpha^{-}$ DC population, likely of myeloid origin.

Finally, it is well documented that the majority of thymic DCs are found in the medulla, and therefore, it is thought that the anatomical location of clonal deletion is at the CMJ or in the medulla. However, we and others have noted the sparse but distinct presence of DC in the cortex of the thymus (See Fig. 1). This often-overlooked observation raises the possibility that cortical DC may be sufficient to mediate clonal deletion. Indeed, we have recently shown that clonal deletion to ubiquitous self-antigens can occur in the cortex, with no involvement of the medulla [76]. Furthermore, we found that thymocytes undergoing this process were preferentially in contact with DC present in the cortex and that conditional ablation of DC significantly impaired clonal deletion of antigen-specific cells. The fact that clonal deletion to ubiquitous self-antigens can occur in the cortex does not preclude the requirement of migration to the medulla and clonal deletion to TRAs. It does, however, expand our thinking as to the mechanism underlying clonal deletion. It is unknown if any phenotypic differences exist between cortical and medullar DC, or if any subset(s) of DC discussed previously reside preferentially in the cortex or the medulla. It was recently shown that most cortical DC are found in close association with small blood capillaries that express CCR7 ligands ([118] and our unpublished data). As positively selected thymocytes also express CCR7, it is interesting to speculate that this exists as an initial mechanism for screening thymocytes for clonal deletion. It also raises the possibility that thymocyte migration to the medulla does not occur randomly but that $CCR7^{+}$ cells travel along blood capillaries, which ultimately lead to the CMJ. Finally, given that cTEC are not efficient at inducing clonal deletion and DC are, it is likely that a second signal that can be delivered by a DC but not a cTEC is necessary to induce apoptosis. We have observed that cortical DCs express B7-2, and it is tempting to think that this is the second signal. However, as discussed previously, there is no absolute role for costimulation by B7-2, although it has been shown that complete deletion of superantigen-reactive cells requires B7-1/2 and CD28 interaction [119]. Therefore, it remains possible that B7-1 and B7-2 play a role in clonal deletion, but it is probable that other costimulatory molecules also exist.

In conclusion, recent advancements have allowed researchers to gain a deeper understanding of the process of clonal deletion. We are finally beginning to understand the molecular pathways that are necessary for the elimination of self-reactive thymocytes, as well as the cellular players that contribute to this process. As the field moves ahead, it is important to develop and use the most physiological tools possible and to consider the intricate anatomical microenvironments of the thymus in order to gain a clear understanding of clonal deletion.

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